AMENDMENT TO THE CLAIMS

This listing of claims replaces all prior versions and listing of claims in the application.

Listing of Claims:

1–169. (canceled)

170. (previously presented) A method for obtaining a modified protein having an improved activity of interest, comprising:

(a) screening a library of clones to identify the presence of a clone having an activity of interest, wherein each clone of the library contains a nucleic acid obtained without selection from a mixed population of organisms from an environmental sample;

(b) subjecting the library to mutagenesis;

(c) expressing the DNA molecules of the mutagenized library to produce one or more proteins; and

(d) screening the proteins to identify a protein having an improved activity of interest compared to the activity identified, thereby obtaining a modified protein having an improved activity of interest.

171. (previously presented) The method of claim 170, wherein the activity of interest is an enzymatic activity.

172–174. (canceled)

175. (previously presented) The method of claim 171, wherein the enzymatic activity is provided by a glycosidase.

176–189. (canceled)

190. (previously presented) The method of claim 170, wherein the clones contain

nucleic acids obtained from extremophiles.

191. (previously presented) The method of claim 190, wherein the extremophiles comprise thermophiles.

192. (previously presented) The method of claim 190, wherein the extremophiles comprise hyperthermophiles.

193. (previously presented) The method of claim 190, wherein the extremophiles comprise psychrophiles.

194. (previously presented) The method of claim 190, wherein the extremophiles comprise halophiles.

195. (previously presented) The method of claim 190, wherein the extremophiles comprise psychrotrophs.

196. (previously presented) The method of claim 190, wherein the extremophiles comprise alkalophiles.

197. (previously presented) The method of claim 190, wherein the extremophiles comprise acidophiles.

198. (previously presented) The method of claim 170, wherein the screening of (a) comprises expression screening.

199–202. (canceled)

203. (previously presented) The method of claim 170, wherein the mutagenesis is via nucleic acid shuffling.

204–213. (canceled)

214. (previously presented) The method of claim 170, wherein the library is generated in a prokaryotic cell.

215. (previously presented) The method of claim 170, wherein the library is generated

in a *Streptomyces sp.*

216. (previously presented) The method of claim 215, wherein the *Streptomyces is Streptomyces venezuelae.*

217. (previously presented) The method of claim 214, wherein the prokaryotic cell is gram negative.

218. (previously presented) The method of claim 214, wherein the prokaryotic cell is a *Bacillus sp.*

219. (previously presented) The method of claim 214 wherein the prokaryotic cell is a *Pseudomonas sp.*

220. (canceled)

221. (currently amended) The method of claim 170, wherein ~~**the library is generated from pooling individual gene libraries generated from the nucleic acids**~~ **prior to step (a), the method comprises the steps of**

(1) isolating organisms from environmental libraries;

(2) extracting nucleic acids from the isolated organisms;

(3) pooling the extracted nucleic acids; and

(4) generating gene libraries from the pooled nucleic acids;

whereby the gene libraries are subject to screening in step (a).

222. (previously presented) The method of claim 170, wherein the library comprises cDNA sequences.

223. (previously presented) The method of claim 170, wherein the library comprises genomic sequences.

224. (previously presented) The method of claim 170, wherein the screening of (a) is by PCR amplification of a nucleic acid sequence of interest using primers

substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest, wherein the primers are labeled with a detectable label.

225. (canceled)

226. (previously presented) The method of claim 170, further comprising comparing the mutated nucleic acid sequence of interest to the non-mutated nucleic acid sequence to identify the nucleotide sequence mutation.

227. (previously presented) The method of claim 226, wherein the comparison is performed using a sequence comparison algorithm.

228. (previously presented) The method of claim 170, wherein the screening of (a) comprises contacting a clone with a substrate wherein interaction of the substrate with the protein expressed by the clone produces a detectable signal.

229. (previously presented) The method of claim 228, wherein the substrate comprises 5-dodecanoylamino fluorescein di-beta-D-galactopyranside (C12-FDG).

230. (previously presented) The method of claim 228, wherein the substrate comprises a first test protein linked to a DNA binding moiety and a second test protein linked to a transcriptional activation moiety.

231. (canceled)

232. (currently amended) The method of claim 170, wherein, prior to (b), the clones are screened for a ~~further~~ desired ~~bioactivity~~ characteristic.

233. (previously presented) The method of claim 170, wherein the library is screened in (a) by contacting a clone of the library with a substrate, wherein a protein produced by the clone is detectable by a difference in the substrate before contact with the clone as compared to after contact.

234. (previously presented) The method of claim 170, wherein the library is normalized before screening the library.

235. (previously presented) The method of claim 170, wherein the nucleic acid of (a) comprises one or more open reading frames.

236. (canceled)

237. (previously presented) The method of claim 170, wherein the improved activity of interest comprises an enhanced or superior enzymatic activity compared to that of wild-type.

238. (previously presented) A method for identifying a protein having an activity of interest, comprising:

(a) incubating nucleic acids obtained directly without selection from a mixed population of organisms from an environmental source with at least one oligonucleotide probe labeled with a detectable label and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

(b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable label;

(c) generating a library from the identified nucleic acid sequences;

(d) screening the library for a specified activity;

(e) mutating a nucleic acid sequence contained in a clone from the library having the specified activity; and

(f) comparing the activity of an expression product of the clone from (e) following mutation with the specified activity of an expression product of the clone without mutation, wherein a difference in the activity is indicative of an effect of introducing at least one sequence mutation,

thereby identifying a protein having an activity of interest.

239. (new) The method of claim 238, wherein the activity of interest is an enzymatic activity.

240. (new) The method of claim 239, wherein the enzymatic activity is provided by a glycosidase.

241. (new) The method of claim 238, wherein the nucleic acids are obtained from extremophiles.

242. (new) The method of claim 241, wherein the extremophiles comprise thermophiles.

243. (new) The method of claim 241, wherein the extremophiles comprise hyperthermophiles.

244. (new) The method of claim 241, wherein the extremophiles comprise psychrophiles.

245. (new) The method of claim 241, wherein the extremophiles comprise halophiles.

246. (new) The method of claim 241, wherein the extremophiles comprise psychrotrophs.

247. (new) The method of claim 241, wherein the extremophiles comprise alkalophiles.

248. (new) The method of claim 241, wherein the extremophiles comprise acidophiles.

249. (new) The method of claim 238, wherein the step of (a) comprises hybridization screening.

250. (new) The method of claim 238, wherein the step of (a) comprises polymerase chain reaction (PCR) screening.

251. (new) The method of claim 238, wherein the step of (a) comprises biopanning.

252. (new) The method of claim 238, wherein the mutagenesis is via nucleic acid shuffling.

253. (new) The method of claim 238, wherein the library is generated in a prokaryotic cell.

254. (new) The method of claim 238, wherein the library is generated in a *Streptomyces sp.*

255. (new) The method of claim 254, wherein the *Streptomyces is Streptomyces venezuelae.*

256. (new) The method of claim 253, wherein the prokaryotic cell is gram negative.

257. (new) The method of claim 253, wherein the prokaryotic cell is a *Bacillus sp.*

258. (new) The method of claim 253, wherein the prokaryotic cell is a *Pseudomonas sp.*

259. (new) The method of claim 238, wherein prior to step (a), the method comprises the steps of

(1) isolating organisms from environmental libraries;

(2) extracting nucleic acids from the isolated organisms;

(3) pooling the extracted nucleic acids; and

(4) generating gene libraries from the pooled nucleic acids;

whereby the nucleic acids from the generated gene libraries are subject to step (a).

260. (new) The method of claim 238, wherein the library comprises cDNA sequences.

261. (new) The method of claim 238, wherein the library comprises genomic sequences.

262. (new) The method of claim 238, wherein the step of (a) is by PCR amplification of a nucleic acid sequence of interest using primers substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest, wherein the primers are labeled with a detectable label.

263. (new) The method of claim 238, wherein the step of (a) is by hybridization of an oligonucleotides substantially complementary to a nucleic acid sequence of interest, wherein the oligonucleotide is labeled with a detectable label.

264. (new) The method of claim 238, further comprising comparing the mutated nucleic acid sequence of interest to the non-mutated nucleic acid sequence to identify the nucleotide sequence mutation.

265. (new) The method of claim 226, wherein the comparison is performed using a sequence comparison algorithm.

266. (new) The method of claim 238, wherein, prior to (e), the nucleic acid sequences are screened for a desired characteristic.

267. (new) The method of claim 238, wherein the library is normalized before screening the library.

268. (new) The method of claim 238, wherein the nucleic acid of (a) comprises one or more open reading frames.

269. (new) The method of claim 238, wherein the improved activity of interest comprises an enhanced or superior enzymatic activity compared to that of wild-type.